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Thermal Stress and the Physiological Response to Environmental Toxicants

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ABSTRACT

Most toxicological and pharmacological studies are performed in laboratory animals maintained under comfortable environmental conditions. Yet, the exposure to environmental toxicants as well as many drugs can occur under stressful environmental conditions during rest or while exercising. The intake and biological efficacy of many toxicants is exacerbated by exposure to heat stress, which can occur in several ways. The increase in pulmonary ventilation during exposure to hot environments results in an increase in the uptake of airborne toxicants. Furthermore, the transcutaneous absorption of pesticides on the skin as well as drugs delivered by skin patches is increased during heat stress because of the combined elevation in skin blood flow coupled with moist skin from sweat. The thermoregulatory response to toxicant exposure, such as hypothermia in relatively small rodents and fever in humans, also modulates the physiological response to most chemical agents. This paper endeavors to review the issue of environmental heat stress and exercise and how they influence thermoregulatory and related pathophysiological responses to environmental toxicants, as well as exposure to drugs.

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INTRODUCTION

Nearly all toxicological and pharmacological studies are performed in resting animals acclimatized to environmental conditions considered ideal for homeostasis /1–2/. Yet, exposure to environmental toxicants and the administration of drugs and other agents can occur under a wide range of environmental conditions in resting and exercising subjects. In view of the effects of temperature and other environmental factors, the physiological response(s) to toxicants is likely to differ markedly from what would be predicted from studies that are performed under standard laboratory conditions. In the interpretation of particular endpoints, toxicologists and pharmacologists only occasionally consider environmental conditions /1–3/.

The often ignored interplay between environmental and chemical/drug responses merits further assessment and review. For example, advancements in the physiology of heat shock proteins and the revolution in physiological monitoring achieved with radiotelemetry (transmission of physiological information to a remote site) have led to a revival in studies that assess how variations in the physical environment influence physiological response(s) to toxicants and drugs. Similarly, heat stress experimentation has traditionally been confounded by a lack of understanding of the effect of experimental manipulation (for example, rectal probes, anesthesia) and environmental conditions (for example, low ambient temperature) on the physiological responses to the heat insult. Heat injury is not only

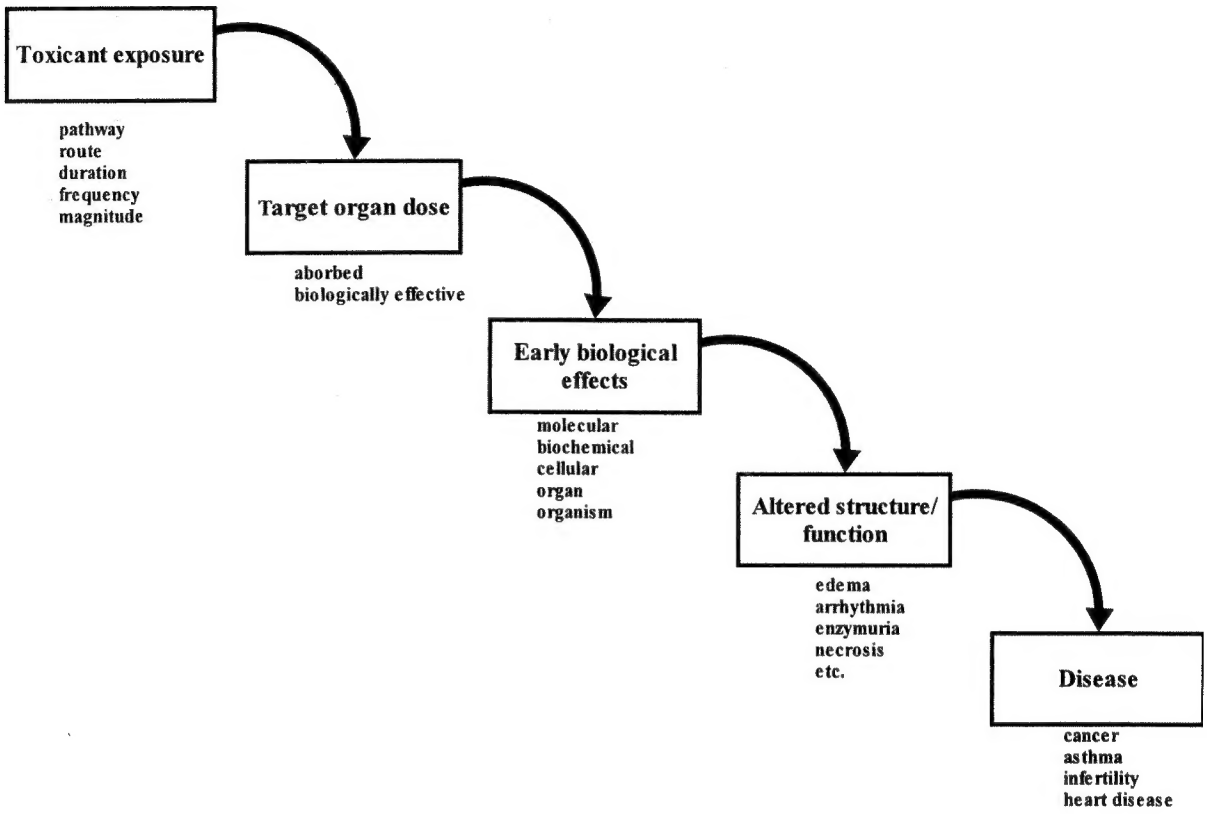

Fig. 1A: An idealistic exposure-dose effect continuum that describes the general biological response and consequences to an environmental toxicant.

a sports /4/ and military /5/ medicine problem but also—as exemplified by the recent high death toll during a heat wave in France /6/—a public health issue that can escalate with global warming /7/. A more thorough understanding of the mechanisms of heat injury and toxicant responses and the potential interactions between the two will aid in the development of more effective strategies to treat exposure to these environmental insults. To this end, this paper endeavors to review the issue of environmental heat stress and exercise and of how they influence thermoregulatory and related pathophysiological responses to environmental toxicants and drugs.

An 'exposure-dose effect' continuum is an ideal starting point for discussing the interaction of heat and other stresses with the physiological response to an environmental toxicant (Fig. 1A). In this scheme, exposure to an environmental toxicant results in an absorbed dose in a given target organ, resulting in early biological effects on systems ranging from the molecular to the organism level. The subsequent altered structure and function of various physiological systems can ultimately lead to a disease state(s). The same scenario is useful for studying environmental heat stress and the thermoregulatory system, as will be discussed here briefly and covered in detail later (Fig. 1B).

Temperature influences the target organ dose of a toxicant in many ways. For example, a higher respiratory rate in a hot environment means greater intake of an airborne pollutant. In homeotherms,

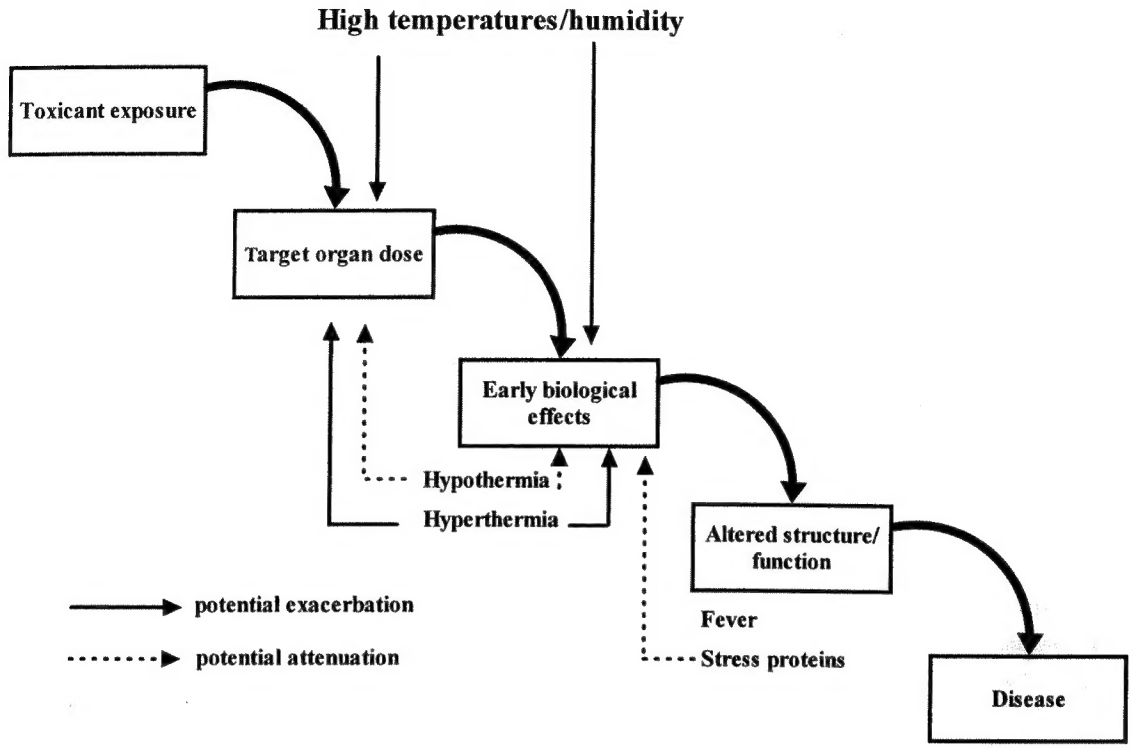

Fig. 1B: Modification of the exposure-dose effect continuum taking into considerations the possible effects and consequences of heat stress and thermoregulatory responses. Exposure-dose effect continuum modified from several sources (see /116/).

whose body temperature is constant and independent of the temperature of its surroundings, the thermoregulatory response to environmental heat stress consists of an increase in skin blood flow and moistening of the skin surface to dissipate core heat to the environment effectively. These physiological responses increase the permeability of skin to many chemicals, resulting in increased cutaneous absorption of potential toxicants and/or drugs. In mammals, the thermoregulatory response to a toxicant can consist of an increase (hyperthermia/fever) and/or decrease (hypothermia) in T_c. Because of the Q_{10} effect—namely, the factor by which the biochemical reaction rate is increased for each 10°C increase in temperature—such profound changes in body temperature can have a direct impact on the toxicity of chemicals. Finally, altered structure and function, such as the expression of stress proteins (heat shock proteins), can influence the damaging effects of the toxicant.

THE PHYSICAL ENVIRONMENT

Environmental temperature, intensity of solar radiation, and humidity are the most important environmental factors governing the geographic distribution, health, and survival of all animal and plant life. Humans, agricultural species, and wildlife encounter a variety of thermal environments that can be relatively mild or extremely stressful (Fig. 2). Living in tropical or sub-tropical areas is associated with hot and humid conditions in the summer and relatively mild winters.

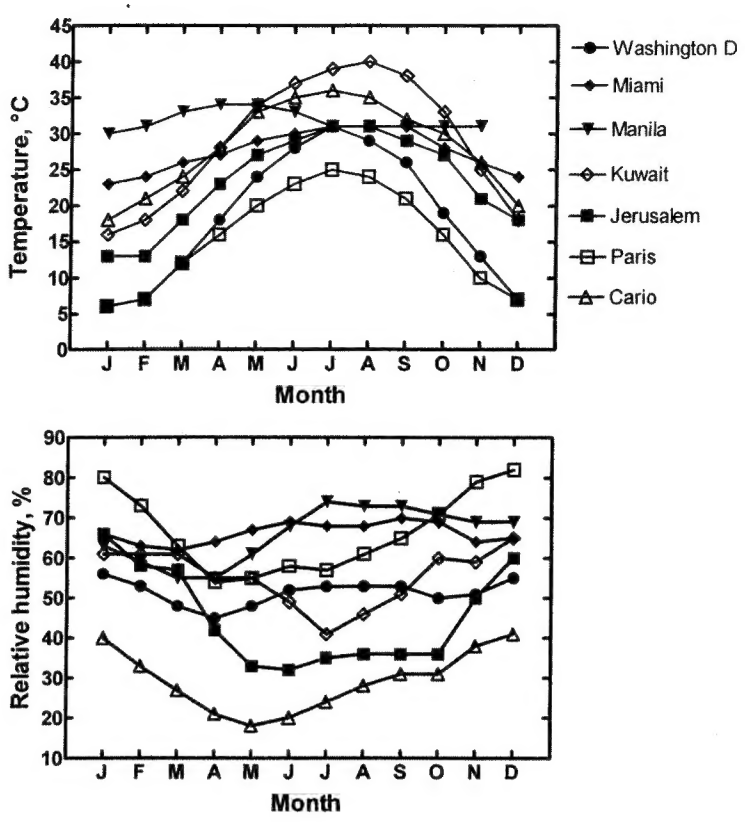

Fig. 2: Average maximum temperature and afternoon relative humidity averaged by month for various cities. Data from BBC weather centre (www.bbc.co.uk/weather)

TABLE 1

Typical environmental conditions for testing toxicological response of rodents and other laboratory species to toxic agents

T_a of 20°–26°C with clean, insulative bedding (temperature dependent on species)
50 percent relative humidity
Still or calm air movement
12:12 light:dark cycle
Fluorescent lighting
Resting, confined conditions with no option for work or exercise
Ad libitum, nutritionally balanced food and water
Filtered air exchanged ~10 times per hour
Sea level

Temperature and humidity in cities such as Manila and San Jose show relative small variations from summer to winter. Inhabitants of temperature zones like in Paris and Washington, DC encounter marked seasonal variations in temperature and humidity, whereas inhabitants of desert regions are subject to extreme hot and dry conditions with intense solar radiation during the day and cold temperatures at night.

Most toxicological and pharmacological studies are performed in experimental animals acclimatized to standard environmental conditions that are ideal for the animal's physiological well being /1–2/. A critical characteristic of the test environment is an ambient temperature associated with minimal strain on the thermoregulatory system (Table 1). The thermoneutral zone is defined as the ambient temperature range equivalent to the minimum metabolic rate and at which the core temperature (T_c) is maintained by nonevaporative physical processes, meaning that T_c is controlled mainly through adjustments in skin-blood flow /8/. Note that the ambient temperature of animal facilities, which typically ranges from 20°–26°C, is below the thermoneutral zone of laboratory rodents (28°–32°C /9/), meaning that their metabolic rate is elevated above basal level. Nevertheless, the metabolic rate depends on several factors, including cage type (for example, metal vs. plastic), bedding, and number of animals per cage. Depending on the material provided, cage bedding has a differential effect on mouse T_c and on metabolic rate /10/. For example, heat-treated wood shavings, which provide the best opportunity to burrow and to reduce heat loss, are associated with a higher daytime T_c of mice /10/. In most cases, rodents are given bedding material that affords insulation and minimizes potential cold stress. Other environmental factors maintained at non-stressful levels include a relative humidity of 50 percent, a 12:12 light:dark photoperiod, still air, *ad libitum* food and water, filtered air, and an atmospheric pressure near to or equal to sea level (see /11/). Test animals are usually sedentary with no option for exercise.

Another important consideration is the period of the circadian cycle during which experiments are conducted. In mammals, a temporal pattern of heat susceptibility and hydration state related to the circadian cycle has been noted /12/. Noteworthy is that heat stress and toxicology experiments are typically performed during the inactive (lights-on or daytime) period in rodents /12–21/. In nocturnal species, whether the T_c or toxicological responses will differ if exposure is initiated during the active (lights-off or night-time) period is not known. Previous data /15/ showing a direct correlation between baseline T_c and mortality would suggest that susceptibility to heat stroke will be elevated during the nocturnal period of rodents (when baseline T_c and activity are elevated), but this hypothesis has not been directly tested. In addition, a study of rodent heat stress and toxicological responses during the nocturnal (active) period would more closely simulate the human condition because elevated ambient temperatures and toxicant exposures are typically encountered during the daytime when humans are most active. Noted species /22/, strain /23/, seasonal /22, 24/, geographic /22–25/ and gender effects /14, 23, 25–28/ on heat susceptibility suggest that the considerable variability of heat and toxicological responses can be influenced by one or all of these factors.

For most rodent research, the so-called ideal environment is not representative of the fluctuation in the natural environment encountered by humans and wildlife on a day-to-day and seasonal basis. As such, most studies do not adequately represent the factors that can have an impact on environmental exposure to toxicants or the physiological responses that can be invoked by the response to such insults. Clearly, alterations in such environmental variables as temperature, humidity, light cycle, and others will alter the physiological response to a toxic chemical. In addition, humans are subjected to varying degrees of work or physical activity on a daily basis, and many such activities are performed while wearing protective clothing that serves as an insulative barrier for heat exchange mechanisms.

Overall, it behooves toxicologists—especially those who are interested in extrapolating experimental data from laboratory animals to humans—to consider how variations in the natural environment will alter toxicological responses.

THERMOREGULATION AND PHYSIOLOGICAL RESPONSES TO ACUTE TOXIC INSULT

For the scope of this review, it is important to provide the salient aspects of the thermoregulatory system and to explain how the system operates when the animal is subjected to a toxic insult. For detailed reviews, the reader is referred to several sources /2, 9, 29–30/. Many researchers, including those not specialized in the field of temperature regulation, are unaware of the effect of different ranges of T_c on the physiological response to toxicological or pharmacological treatment. Typically, little or no consideration is given to the impact of measurement techniques on thermoregulatory mechanisms. Most researchers are unaware that the physical handling associated with an experimental design can have an impact on T_c and, consequently, on the response to a toxicant or heat stress. Typically, investigators attempt to maintain T_c as a constant in an experimental design such that temperature does not have to be considered in the analysis of the data. To this end, researchers will clamp the T_c of the test subject throughout the study. This approach can be a mistake because, as will be shown below, changes in T_c often represent adaptive responses aimed at improving survival to such stressful stimuli as toxicants, drugs, and heat exposure. Similarly, a consideration of the normal baseline T_c of different rodent species is often ignored. As described previously, environmental conditions can have a profound influence on T_c. We anticipate that the use of radiotelemetry, which eliminates many of the experimental stressors associated with handling and restraint, will alleviate many of these previously unrecognized confounders. Nevertheless, environmental and physical stressors (for example, exercise) can have a similar impact on the physiological responses to environmental stressors. For example, researchers have recognized that initial (baseline) T_c can be a predisposing factor to heat stroke death; that is, a higher baseline T_c is associated with enhanced heat-stress susceptibility /15/. The data indicate that heat susceptibility must be regarded in the context of additional environmental or experimental stressors that can be present at the time of the heat insult. For example, exercise or toxicant exposure can induce significant increases in T_c that will affect a subsequent response to heat exposure. Similar interactive and potentially synergistic effects of heat and toxicant exposure are expected to exist and should be considered.

Thermoregulatory System

The neurological and pharmacologic characteristics of the thermoregulatory system have been extensively reviewed /2, 29, 31/. The thermoregulatory system of mammals has evolved to maintain a stable internal (namely, core) temperature over a relatively wide range of ambient temperatures Temperature regulation is fundamentally based on the core heat balance equation, which is a mathematical expression of the rate at which a subject generates and exchanges heat with its environment:

$$S = M - (W) - (E) - (C) - (K) - (R)$$

where S is the rate of heat storage in the core (positive for increase in core heat content), M is metabolic rate, W is work rate (positive for useful mechanical power accomplished; negative for mechanical power absorbed by core); E is evaporative heat transfer (positive for evaporative heat loss; negative for evaporative heat gain); C is convective heat transfer (positive for heat transfer to the environment; negative for heat transfer to core); K is conductive heat transfer (positive for heat transfer to environment; negative for transfer

TABLE 2

The Heat Stress Continuum[1]

Term	Definition
Heat Cramps	Intermittent cramping pain in muscles subjected to strenuous activity; normal T_c; may occur in cold environment; treated with rest and sodium/potassium/fluid replacement
Heat Exhaustion (Heat Prostration; Heat Collapse)	Heat illness due to salt or water depletion resulting from strenuous physical exercise or prolonged exposure to a hot environment; T_c may or may not be elevated; decreased cardiac output
Heatstroke	$T_c > 40°C$ and CNS abnormalities (delirium, fainting, seizures, and coma) that result from prolonged exposure to a hot environment (classic) or strenuous physical exercise (exertional)

[1] (adapted from Petersdorf et al. /38/)

to core), and *R* is radiant heat exchange (positive for heat transfer to environment; negative for transfer to core). The dimensions are in Watts (W), a measure of heat flow. Nevertheless, the equation terms are often expressed in units normalized to surface area (W/m^2) or core mass (W/kg).

The thermoregulatory response to a stressful insult (environmental, bacterial, or viral) consists of hyperthermia and/or hypothermia, as described above. The regulated nature of the imposed T_c response is representative of its survival value. *Regulated hyperthermia*, or fever, functions as a survival mechanism during infection by inhibiting pathogen replication and growth. On the other hand, environmental heat stress can lead to *unregulated hyperthermia* that can be harmful to the organism (see section on the *Heat-illness continuum*).

Thus, in response to high environmental temperature, physiological and behavioral mechanisms are used to inhibit heat production and to stimulate heat loss for the purpose of maintaining T_c within an optimal range for physiological functioning. Conversely, *regulated hypothermia* represents the opposite extreme of the thermoregulatory continuum as a fever that serves a protective function following exposure to several stressful stimuli, including hypoglycemia /32/, hemorrhage /33/, dehydration /34/, and infection /35–36/. (See section on *Integrative Thermoregulatory Responses to Toxicants and Heat Exposure*).

Heat-illness continuum. Heat-illness syndromes are typically described as discrete events but are best regarded as a continuum of increasing severity (Table 2).

Heat cramps, precipitated by strenuous muscle activity and profuse sweating resulting in a loss of electrolytes, are the most benign condition. In this condition, spasms of skeletal muscles in the extremities can be sporadic but painful /37/. Heat cramps are not associated with elevated environmental temperatures but typically occur following exercise in the cold /38/.

Heat exhaustion (also referred to as heat prostration or heat collapse) is the most common heat syndrome and is associated with water or salt depletion in a hot environment. This mild-to-moderate illness is associated with an inability to maintain adequate cardiac output, resulting in an elevation in T_c and the potential for collapse. The use of diuretics and other medications can predispose such individuals to heat exhaustion.

Heat stroke, the most serious heat syndrome resulting from prolonged exposure to a hot environment, is the focus of this review. The clinical definition of heat stroke includes the following:

- T_c in excess of 41.0°C (often referred to as *hyperpyrexia*),
- hot, dry skin, and
- central nervous system (CNS) dysfunction, such as delirium and convulsions.

The absence or presence of an exertional component during heat exposure allows further classification of heat stroke into its classic (namely, passive) or exertional forms.

- *Classic heat stroke* results from passive exposure to a hot environment. *Passive heat stroke* is typically observed in immunocompromised and aging populations, which show enhanced mortality during heat waves /6, 39–40/. Pre-existing conditions, such as mental illness, alcoholism, or drug use (for example, diuretics, anticholinergics) can predispose individuals to classic heat stroke /40–41/.
- On the other hand, *exertional heat stroke* occurs in healthy, young individuals who are undergoing strenuous physical activity in a hot environment. Athletes and soldiers represent two high-risk populations for this form of heat illness, although heat acclimatization can reduce the risk of injury in such a population /42/. Exertional heat injury is a particularly complicated heat syndrome to study because dissociating the direct effects of strenuous physical activity from that imposed by exposure to a hot environment is difficult.

Experimental heat illness. The thermoregulatory system has evolved rapid responses to correct for short-term imbalances in heat exchange and long-term responses that develop with prolonged exposure to heat or cold stress (Fig. 3). Mammals utilize autonomic and behavioral thermoeffectors

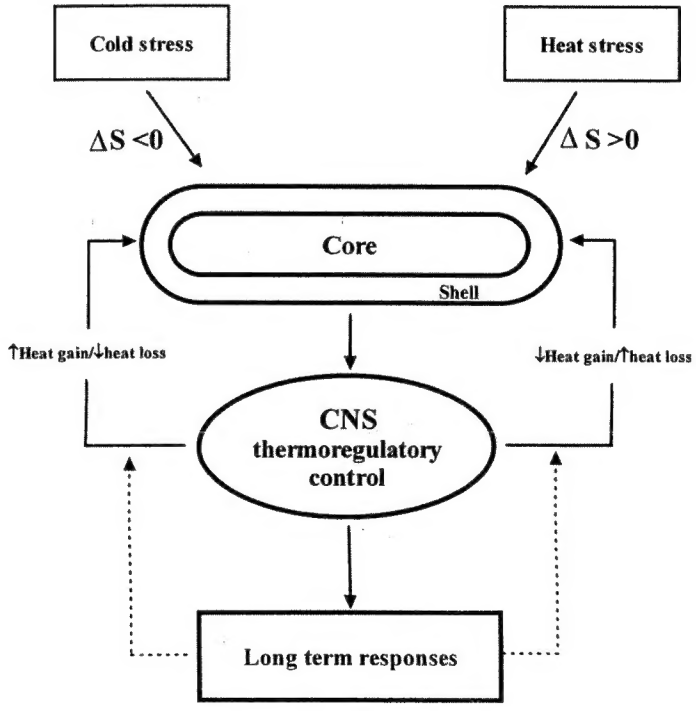

Fig. 3: Rapid and adaptive responses of the thermoregulatory system to heat and cold stress. An elevation in temperature of the shell and/or core (S>0) results in a rapid thermoeffector response to increase heat loss; a reduction in shell/core temperature (S<0) activates the heat gain/reduction in heat loss loop. The long-term adaptive responses develop with continued thermal stress.

to alter heat exchange to a thermal (heat or cold) challenge, thus maintaining homeostasis. In response to prolonged heat exposure, several biochemical, physiological, and morphologic adaptations occur ultimately to decrease the energy requirements of the thermally challenged organism. For example, when exposed to an ambient temperature above their thermoneutral zone, rats increase the rate of heat loss through peripheral vasodilation, increased respiration, and behavioral spreading of saliva on the fur surface.

During prolonged heat exposure and in the absence of a constant water source, evaporative heat loss due to salivary spreading can result in significant dehydration of the animal. With repeated exposure to the thermal environment, however, biochemical adaptations occur to diminish metabolic demands, to minimize internal heat production, and to lower the requirements for evaporative water loss. Heat acclimatization induces morphological changes, such as reduced fur thickness and increased vascularization of the peripheral tissues that facilitates heat dissipation. In rodents, which use salivary spreading as the main source for evaporative cooling, acclimatization induces an increase in the size of the submaxillary gland /43–44/. This morphologic change results in a longer duration and a larger volume of secreted saliva compared with that observed in non-acclimatized rats.

In humans, improved sweating and skin blood-flow responses, lowered metabolic rate, and improved fluid balance serve to enhance thermal tolerance /45/. As expected, such adaptations serve to decrease the hyperthermic plateau, allowing a conservation of water balance and enhanced survival.

Integrative Thermoregulatory Responses to Toxicants and Heat Exposure

Several reviews have been published on the acute and delayed effects of toxic chemical and heat exposure on the thermoregulatory system of experimental animals and humans /2, 46–48/. The use of radiotelemetry and other systems to monitor the behavioral thermoregulatory responses of unrestrained rodents has led to a better understanding of the effects of various classes of pesticides, other toxicants, and heat stress. In general, the exposure of rats and mice to such toxicants as metals, ozone, solvents, anti-cholinesterase (ChE) insecticides, or heat stress induces a hypothermic response at ambient temperatures below the thermoneutral zone, namely, $< 28°C$ for rats and mice /2, 18, 21/. The hypothermic response will persist for several hours after the initial exposure. As recovery progresses, an increase in core temperature is often seen, such as that occurring after exposure to anti-ChE insecticides (see below). The elevated temperature can persist for several days after exposure. Hyperthermia is also observed in response to toxicants when animals are housed at an ambient temperature within or above the thermoneutral zone /2/.

Interestingly, the thermoregulatory response to environmental toxicants is similar to that observed in mice during recovery from acute heat exposure (Fig. 4). Most likely, similar physiological mechanisms are mediating the responses to the different environmental insults. Although several characteristics of the thermoregulatory response during direct heat exposure are well defi ned in animal models, the response observed during recovery has received less attention. This state of affairs is rather surprising because changes in T_c during recovery from heat exposure can provide powerful insight into the pathophysiological changes that occur as a direct result of the initial heat insult.

Several studies have reported hypothermia as a common thermoregulatory response to prolonged heat exposure /18–19, 21/, with the magnitude and duration of the response being directly related to the severity of the heat insult /21, 49/. How T_c is affected following exposure to a combination of heat and toxicant exposure is currently unknown. Most likely the response will be at least partially dependent on the timing of exposure to the two

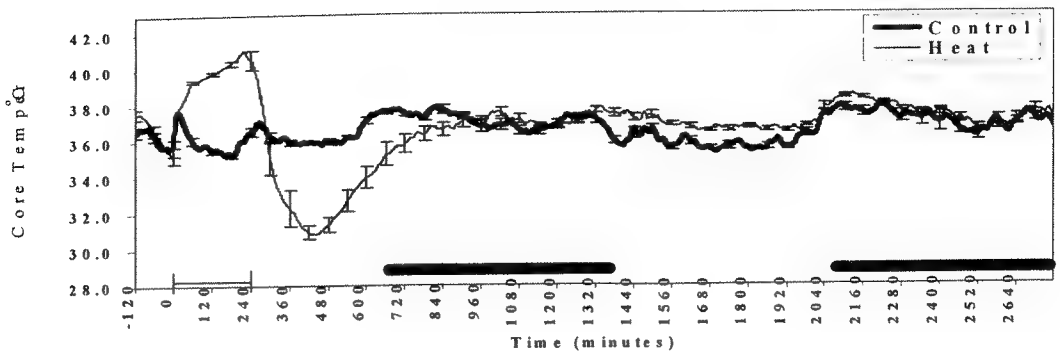

Fig. 4: Time-course of core temperature of C57BL/6J male mice monitored by radiotelemetry following acute heat stress. Mice were heated at an ambient temperature of 39.5±0.2°C, in the absence of food and water, to a T_c of 42.4°C and then returned to an ambient temperature of 25±2°C for ~48h of recovery. Note initial hypothermia followed by elevation in daytime T_c the day following heat stress. N = 11–12 per treatment group.
▬ lights-off or active period on a 12:12h L:D cycle. Modified from Leon et al. (2005).

insults (for example, simultaneous or sequential exposure). If a similar relation between heat severity, toxicant exposure, and T_c responses exists for humans, then this parallel could have important clinical implications for the treatment of those patients for which specific details regarding the timing of an environmental insult(s) are not known.

The hypothermic response during recovery from toxicant or heat exposure has commonly been regarded a failure of the thermoregulatory system rather than a regulated response aimed at survival. Meeter and colleagues /115/ noted that the hypothermic response to organophosphate-based nerve gas agents in the rat is associated with an elevation in tail skin temperature (the tail is an important thermoregulatory organ in the rat), suggesting an active thermoregulatory response to regulate T_c at a lower level. Using a temperature gradient that allows rodents to behaviorally select a wide range of ambient temperatures, the acute hypothermic response to a variety of toxic agents was shown to be accompanied by a preference for cooler ambient temperatures /2, 9, 46/. The preference for cooler ambient temperatures concomitantly with a decrease in T_c provides further evidence of an active thermoregulatory response to a decrease in core temperature—a response coined *regulated hypothermia*. If the toxicants were impairing the thermoeffectors for heat gain and heat loss without affecting the CNS control mechanisms, then one would expect the rodent to select warmer temperatures in the gradient and reverse the hypothermic effects of toxicant exposure. This response would be termed *forced hypothermia* but is rarely reported in rodents dosed acutely with toxicants /2/.

Core Temperature and Survival to Toxicants and Heat Exposure

The thermoregulatory response to toxicants and heat exposure that results in a lowering of T_c is likely an adaptive response to improve recovery and survival. The generally increased sensitivity of homeothermic and poikilothermic[1] species to drugs and chemicals with an increase in temperature has

[1] having a body temperature that varies with the temperature of its surroundings

been recognized since the first half of the twentieth century /2/. Fish and amphibians were often used as test species in toxicological studies, and the profound impact of water temperature on the toxicity of drugs and toxic chemicals was quickly recognized. Rats, mice, and other homeothermic test subjects are capable of thermoregulating against a wide range of ambient temperatures but nonetheless show a greater sensitivity to a variety of toxicants when exposed to heat stress.

The toxicity of most chemicals increases with temperature because the mechanisms of toxicity have a $Q_{10} > 1.0$. That is, the molecular and cellular processes of toxicity, such as lipid peroxidation, formation of reactive oxygen species (ROS), and the disruption of membrane permeability accelerate with a rise in temperature. Exceptions to this rule include two major categories of insecticides—pyrethroids and DDT. The toxicity of these chemicals increases with a reduction in temperature (namely, $Q_{10} < 1.0$) because their mechanism of toxicity, opening sodium channels on nerve membranes, is exacerbated with cooling /50/.

Overall, small rodents have a relatively large surface area:body mass ratio and are capable of rapid cooling when challenged with acute exposure to a variety of toxicants, drugs, and heat. Hypothermia appears to be an adaptive response that attenuates chemical toxicity by slowing the rate at which the chemical exerts its toxic effects. Although the precise mechanisms are not known, hypothermia also shows protective effects against heat exposure in that it is correlated with an attenuation of organ damage /49/. Because housing at an ambient temperature that prevents the development of hypothermia induces a significant increase in heat-stress mortality also speaks to the adaptive value of this thermoregulatory response /21, 49/. Nevertheless, the applicability of these findings to the human condition currently remains unrecognized. With an increase in body mass and the resultant decrease in the surface area:body mass ratio, a physical limit is imposed on the ability of an organism to cool rapidly. Although housing at a relatively low ambient temperature will facilitate the development of hypothermia, the marked hypothermia seen in rodents following toxicant and heat exposure is rarely observed in adult humans or in other large mammals. Such nonconformity in the T_c responses among species could be due to body scaling issues and/or to clinical interventions that have masked the response.

The remainder of this review will focus on the specific effects of environmental heat exposure and the physiological response to chemical toxicants and drugs. Both passive and work-induced hyperthermia represents significant stressors to humans and other species. Not only are groups of the general public susceptible but also members of the armed services are particularly susceptible when one considers the high-intensity sustained military operations in desert regions. Heat, mandatory protective clothing for chemical and biological warfare, and stress of military life can combine to exacerbate the adverse effects of heat exposure.

Reviewing this topic in light of the possible impact of the greenhouse effect is also pertinent. As energy demands increase, much of which is required for air conditioning to maintain an ideal level of thermal comfort, the toxicants generated by burning fossil fuels combined with warmer air temperatures from the greenhouse effect are likely to exacerbate the health effects of airborne toxicants from fossil fuels as well as from other sources.

Heat Loss Thermoeffectors: Effect on Toxicant Intake

Chemicals can enter the core via three principal routes: respiratory surfaces, gastrointestinal tract, and skin /51/. Interestingly, the efficacy by which toxicants enter the body is influenced by thermoregulation because the enhanced activity of some thermoeffectors influences the rate of entry of toxicants through the cutaneous and respiratory routes (Fig. 5). The surfaces of the respiratory tract and skin are integral for the operation of thermoeffectors for evaporative and dry heat loss.

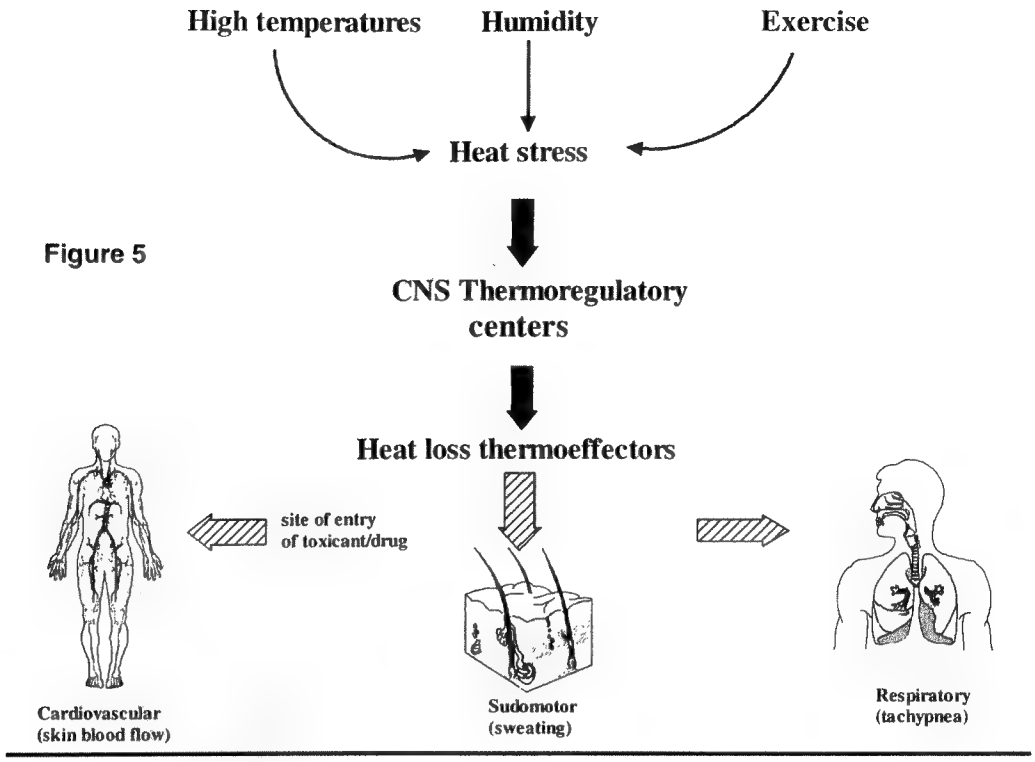

Figure 5

Fig. 5: Depiction of how thermoeffectors for heat dissipation, peripheral vasodilation, sweating, and respiration, can modulate the entry of certain types of toxicants and drugs into the core

The thermoregulatory system responds to heat stress and exercise by activating three key systems to dissipate excess heat: cardiovascular, respiratory, and sweating (vasomotor and sudomotor). The combination of peripheral vasodilation to increase skin blood flow and to raise skin temperature along with sweating provides an effective mechanism to dissipate a heat load /29/. Hence, when a homeotherm is in an environment in which it must actively dissipate heat, the subject is likely to be more susceptible to lower doses and/or to the concentrations of a toxicant.

On the other hand, in a cold environment, the increased demand for heat production results in an elevation in the respiratory rate, which can increase the intake of airborne toxicants and raise the susceptibility. Homeotherms exposed to cold temperatures also consume more food, which raises the possibility of increasing the intake of contaminated food. This scenario has been documented in wild rodent populations that in the winter feed more on plants with natural toxins /52/.

When heat stressed, a true panting animal exhibits a marked increase in breathing frequency. Non-panting homeotherms, including humans and rodents, also exhibit an increase in breathing frequency and minute volume that contributes to a relatively modest increase in evaporative water loss as compared with that of a panting animal. In rodents, the situation is slightly different because they do not sweat but rather rely on the behavioral spreading of saliva on the fur surface to enhance evaporative cooling. This behavioral adaptation to heat will manifest as an increase in overall activity

and metabolism. In rodents, increases in locomotor activity due to escape or avoidance behavior are also noted in response to prolonged heat exposure /53/. Increased activity will stimulate an increase in metabolism, T_c, and respiratory rate and can result in enhanced exposure to toxins. Similarly, an added heat load from exercise in the human condition will increase ventilation and augment the total intake of airborne pollutants /54/.

PHARMACOKINETICS OF TOXICANTS AND DRUGS

In heat-stressed humans and in certain other mammals, sweating is the principal thermoeffector response. In humans exposed to warm temperatures, t he eccrine sweat glands are activated by cholinergic pathways. The redistribution of warm blood from the core to the surface, combined with evaporative cooling from sweating, is an effective means of dissipating excess core heat. On bare skin, the combination of moisture and warm temperature provides an ideal environment for accelerating the dermal absorption of many types of drugs and pesticides /55–56/. Hence, the thermoregulatory response to heat or cold stress is expected to have an impact on the transcutaneous absorption of environmental toxicants, as well as on transdermal drug therapy.

Environmental Toxicants

The results of in vitro and in vivo studies suggest that during heat stress and/or exercise, the activation of thermoeffectors to dissipate heat will accelerate pesticide absorption in humans. An in vitro model has been used to show how blood flow, temperature, and relative humidity affect the absorption of parathion, an anti-ChE pesticide. A small section of porcine skin positioned over a flow-through diffusion cell provides an ideal means for controlling air temperature, relative humidity, perfusate temperature (an indication of T_c), and flow of the perfusate (an indication of the potential effects of blood flow) while studying the percutaneous absorption of a pesticide /57/. The absorption of radiolabeled parathion across porcine skin increases dramatically with elevations in air and/or perfusate temperature. For example, a 5°C increase in air and perfusate temperature leads to more than a twofold increase in parathion absorption. Possibly, skin warming can raise lipid fluidity and the permeability of the dermal tissues, leading to the increased penetration of the pesticide. The cutaneous absorption of parathion is affected by relative humidity and perfusate flow. The effects of humidity are profound, suggesting that increased moisture on the skin increases its permeability to parathion. As parathion is a lipophilic molecule, why percutaneous absorption would increase with additional moisture on the skin is thus not clear. Noteworthy is the marked effect of humidity in the pig, a non-sweating animal. One can only surmise that species that sweat to thermoregulate in the heat would be especially susceptible to pesticide absorption in a warm and humid environment.

Organophosphorus (OP) compounds are potent neurotoxic chemicals that are widely used in medicine, industry, and agriculture. A few studies in human subjects have shown how perspiration accelerates the cutaneous absorption of OP agents. Human volunteers were exposed to ambient temperatures of 14°, 21°, 28°, and 40.5°C while their hands and arms were exposed to a 2 percent parathion dust for 2 hours /58/. The absorption of the insecticide was estimated by the quantity of paranitrophenol, a metabolite of parathion, excreted in the urine. The dermal absorption of parathion was mildly affected at low temperatures and markedly affected at warm ambient temperatures; absorption increased by 25 percent when the temperature of exposure was raised from 14°–21°C; but from 21°–28°C, however, parathion absorption increased by just 17 percent. Raising the ambient temperature from 28° to 40.5°C led to a 180 percent increase in absorption /58/. Although the rate of sweating was not quantified, clearly the

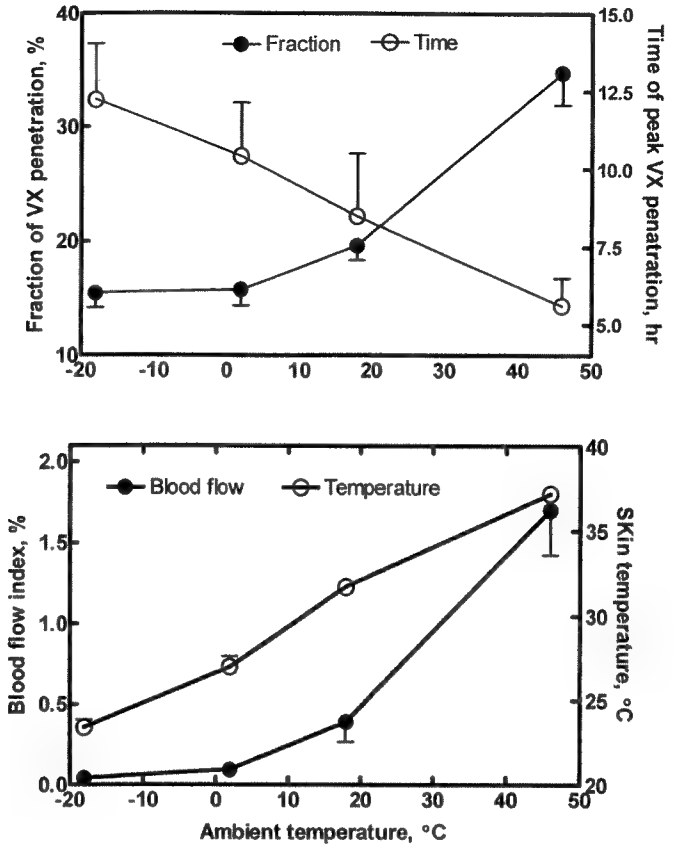

Fig. 6: **A.** Effect of ambient temperature on the rate and time to peak absorption of the OP nerve gas agent VX in human volunteers. Absorption fraction estimated on rate of inhibition of red blood cell cholinesterase activity.
B. Relation between ambient temperature and skin blood flow and skin temperature in untreated human volunteers. Data taken from Craig et al. (1977).

subjects perspired profusely at the warmest ambient temperature. Also noteworthy is the increase in parathion absorption at lower ambient temperatures, despite a lack of sweating. The warmer skin temperature is likely to be a critical factor affecting parathion absorption even without sweating. The dose of parathion was relatively low because red blood cell and plasma ChE activity were unaffected by the treatment. In another study on human volunteers, small amounts (3–8 µg/kg) of the nerve gas VX (S-(2-di-isopropylaminoethyl) O-ethyl methylphosphonothiooate) were applied topically to their cheeks and forearms at ambient temperatures of –18°, 2°, 18°, or 46°C (Fig. x6). The VX remained on the skin for 3 h, and its penetration into the core was estimated by measuring the inhibition of in red blood cell ChE activity /59/. The transcutaneous absorption of VX was directly dependent on ambient temperature. Skin temperature and skin blood flow varied directly with ambient temperature. Overall, the penetration across the cheek was far more effective that in the forearm. The authors postulated that after exposure to VX or a comparable agent,

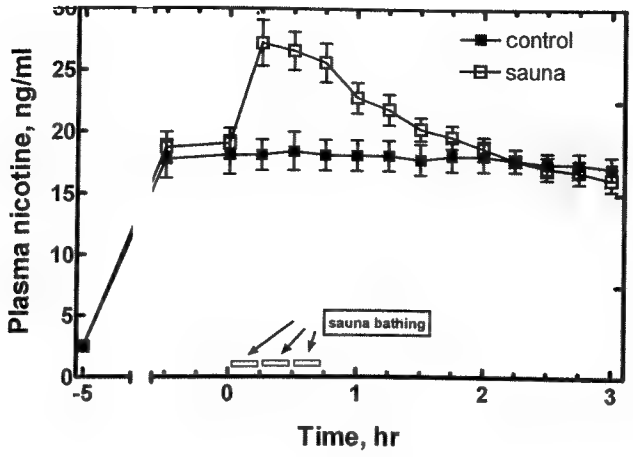

Fig. 7: Time-course of plasma nicotine levels in humans wearing transdermal nicotine patches (25 mg/16 hour) and exposed to a sauna (three 10 minute sessions, 77°–84°C, 26–32 percent relative humidity) starting 5 hours after patches were applied. Modified from Vanakoski et al. (1996).

cooling the skin would delay absorption, thus allowing for safer decontamination of an exposed subject. As the sweat glands are activated by cholinergic stimulation, parathion and other anti-ChE pesticides should directly stimulate sweating through the inhibition of ChE activity. Hence, a warm and humid environment should be expected to exacerbate the adverse effect of a dosage from exposure to an anti-ChE pesticide because of the cholinergic stimulation of sweating combined with greater transcutaneous absorption across moist skin. The authors also noted that subjects exposed to parathion at the highest ambient temperature continued to exhibit sweating from the exposed area of skin for several days after decontamination /58/.

Transdermal Drug Absorption

The transdermal administration of drugs is considered an ideal means of maintaining steady-state levels of a drug in the circulation while minimizing the wide fluctuations commonly seen with oral dosing. Nevertheless, the transcutaneous delivery of a drug with a skin patch can be closely tied to the activation of thermoeffectors for heat dissipation—sweating and peripheral vasomotor tone. As with the OP toxicants discussed above, transcutaneous drug delivery is directly related to the amount of moisture on the skin. Transdermal absorption of a drug would no doubt be dependent on skin blood flow as well. Hence, one should consider how heat stress and exercise would modulate transcutaneous drug therapy. Furthermore, alterations in the thermoregulatory capacity of aged individuals, under both control and heat stress conditions, will undoubtedly have an impact on the efficacy of transcutaneous drug therapy in this population. As the aged population increases over the ensuing decades, this aspect will become an increasingly important consideration.

Several studies have shown that a warm and hot environment augments cutaneous drug absorption. In one study, the transdermal administration of clonidine in human subjects led to significantly higher plasma concentrations of the drug when the patch was worn during the summer than it did when worn in the winter /60/. Nicotine patches are one of the most heavily used forms of transdermal drug therapy. The subjects wearing the patches are likely exposed to a wide range of environmental conditions. Vanakoski et al. /61/ showed that during exposure in a sauna, human subjects with nicotine

patches sustained greater peak elevations in nicotine (Fig. 7). In subjects wearing nicotine patches and exposed to three 10-minute sessions in a sauna, the peak rise in plasma nicotine was 145 percent above that of controls not exposed to heat stress. Transdermal absorption of the cardiac drug glyceryl trinitrate (GTN) was increased by either having the subjects exercise or exposing them to a sauna /62/. In subjects who exercised on an ergometer, plasma levels of GTN were twice that of controls and nearly fivefold higher in subjects who spent 20 minutes in a sauna. Considering seasonal effects on blood pressure, related cardiovascular functions, and human mortality /1/, an urgent need exists for a better understanding of physiological responses to heat and cold and their effects on the pharmacokinetics of drug patches.

PHYSIOLOGICAL RESPONSES TO TOXICANTS AND HEAT STRESS

Heat Shock Proteins

Heat shock proteins (HSPs) are phylogenetically conserved proteins that function as molecular chaperones, preventing the misfolding and aggregation of proteins under stressful conditions. Whereas certain forms of HSPs are constitutively expressed, others are induced in response to environmental (heavy metals, heat stress), physiological (cell differentiation), and pathological (infections) stimuli /63/. Heat shock—namely, raising the T_c of an organism to near lethal levels for a brief period—is commonly used to elicit the expression of HSPs. Hypoxia, reperfusion of ischemic tissues, and exposure to toxic chemicals represent additional HSP-inducing stimuli.

In normal individuals, HSPs are present extracellularly and in the circulation /64–65/, although the function of circulating HSPs under non-stressful conditions is unknown. Such proteins are found in all organisms, from bacteria to humans, and are thought to have a major role in providing cytoprotection in the face of exposure to a variety of stressful insults /66–67/. Heat-shock-protein expression was originally observed in heat-shocked *Drosophila melanogaster*. Following an episode of heat shock, an organism like *Drosophila* has a greater tolerance to heat, an effect attributable to the increased expression of HSPs.

Most research on the cytoprotective role of HSPs has been carried out using in vitro systems, although in vivo approaches have also successfully demonstrated the functional role of HSPs in cytoprotection from heat and other stressors. Heat shock—for example immersing anesthetized rats in a hot water bath for 15 minutes to increase their T_c to 42°C—will induce an increase in CNS HSP72 within 4 hours /68/. Heat shock protein levels peak between 8 and 16 hours and return to baseline at 48 hours. Unanesthetized rats exposed for 5 to 10 minutes to a hot ambient temperature of 40°C, thereby producing a T_c of 42°C, is sufficient to evoke HSP induction in the liver /20/. In mouse liver, heat shock of this nature improves the response to a toxic dose of amphetamine /69/.

Although such acute heating episodes are extremely interesting responses, one must wonder about the relevance of HSP expression to the types of stress that are typically encountered in nature. We now know that such a heat shock stressor is not needed to evoke the expression of HSPs in mammals. Heat acclimation for several weeks to a hot but not lethal environment is sufficient to elicit significant expression of HSPs (Fig. 8). For example, rats maintained for 4 weeks at an ambient temperature of 34°C undergo a 175 percent increase in HSP72 levels in cardiac tissue /70/. The degree to which core or peripheral tissue temperatures must increase to elicit the HSP response is not clear. Although one would expect a significant elevation in T_c when a rat is maintained continuously at 34°C, this parameter is commonly not measured in such studies /70/.

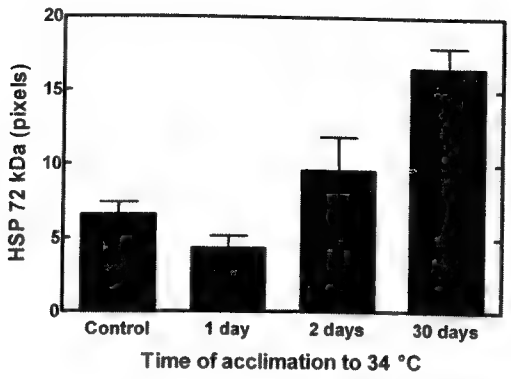

Fig. 8: Change in level of heat shock protein (HSP 72) in rat myocardium during the course of acclimation to a warm environment. Data from Maloyan et al. /70/.

The physiological and molecular responses to heat acclimation can affect the efficacy of drugs and toxicants. The laboratory of M. Horowitz has published a series of studies on the cardioprotective effects of heat acclimation (for review, see /71/). Following heat acclimation, rats undergo a reduction in heart rate and an elevation in stroke volume, which together lead to increased cardiac efficiency. In the heat-exposed rat, intrinsic changes in the cardiac muscle match the function of the heart to the peripheral vascular load, thereby providing the animals with improved heat tolerance. Such processes can be relevant to drugs and toxicants having their primary effect on the heart. Relevancy to other organ systems can also be assumed under such conditions.

The myocardium of the heat-acclimated rat is more resistant to hypoxia and ischemia than that of non-acclimated rats. This protection is thought to be attributable in part to the expression of HSPs. Following ischemia, infarct size is significantly smaller in a heat-acclimated heart than in a non-acclimated heart. Heat acclimation also increases the level of ROS scavengers, a response that would also provide protection to toxic insults. Compared with non-acclimated rats, the latency to hyperbaric oxygen-induced convulsions in the heat-acclimated rat is twice as long, a response accompanied by a marked increase in CNS levels of HSP72 /72/. Hyperbaric oxygen is toxic and causes an increase in ROS formation. Following deacclimation in a 24°C environment, rat HSP72 levels dropped over a 4-week period, accompanied by a reduced latency to oxygen toxicity. The results of these studies suggest that several weeks of acclimation to a warm temperature expected to cause mild elevations in temperature of core and peripheral tissues is sufficient to evoke HSP expression.

The HSP response, coupled with other cellular and molecular changes that are associated with heat acclimation, protects the animal from insults that damage cells through oxidative stress. From this information, one would expect heat-acclimated animals to be more tolerant to many drugs and toxic chemicals.

Gastrointestinal Permeability and Endotoxin

In addition to the primary function of the gastrointestinal tract in the digestion and absorption of food, the mucosa of this organ also serves as a functional barrier against exposing the host to toxins and microorganisms that are found in the intestinal lumen. Increases in intestinal mucosal permeability can be detrimental to the host by contributing to multiple organ failure and mortality

following response to injury. Increased intestinal barrier dysfunction is a common pathophysiological response to such severe trauma as hemorrhage /73/, sepsis /74/, inflammatory bowel disease /75/, exercise /76/, and heat exposure /77/. During exercise and heat exposure, gastrointestinal barrier dysfunction results from a reduction in blood flow because a greater proportion of cardiac output is shunted to the skin surface to dissipate core heat. The resulting reduction in splanchnic blood flow produces hypoxia and oxidative and nitrosative stress, impairing tight junction integrity and thus increasing epithelial permeability /77–79/. The resultant leakage of toxins and endotoxin from the intestine lumen can have severe pathophysiological consequences. Endotoxemia is a reported factor in heat stroke deaths in humans /80–81/.

One hypothesis states that the systemic inflammatory response ensuing after heat exposure and the resultant endotoxemia is responsible for many pathophysiological complications associated with heat injury, including the presence of delayed fever. Thus, in addition to the immediate effects of heat stress per se, one also has to consider the sequelae of physiological changes ensuing after the initial heat insult. Following heat exposure, endotoxemia, or toxicant exposure, many biochemical responses overlap, such as the production of endogenous cytokines /80, 82/. This aspect can partly explain how predisposing factors, such as arteriosclerotic disease or inflammatory bowel disease, can enhance an individual's susceptibility to heat stroke. Also predicted is that the responses will have a significant impact on the response to toxicant exposure, although this notion has not been examined.

HUMAN MORTALITY, TOXICANT EXPOSURE, AND TEMPERATURE

The possibility of an interaction between the exposure to air pollutants, human mortality, and thermal stress has been a topic of several epidemiologic studies /83–86/. Although aged humans have a baseline T_c similar to that of young adults, their thermoeffector mechanisms show various deficits /87/. In general, aged humans have deficiencies in the ability to reduce skin blood flow and to elevate heat production when subjected to cold stress and an impaired ability to raise sweat output and skin blood flow when subjected to heat stress. In view of the deficits in thermoeffector function, an increased incidence of deficiencies in thermal homeostasis in the aged during periods of thermal stress is not surprising (for review, see /86/). The interaction among season, thermoregulatory function, and the health of the aged and other susceptible groups behooves one to consider how sensitivity to pollutants and other toxicants can vary as a function of seasonal change and aging.

The association between extreme changes in environmental temperature and mortality would be expected in view of the deficiencies in thermoeffector function in the aged. Indeed, high rates of death and sickness during summer heat waves and winter cold snaps receive considerable attention in the media. On the other hand, one should also wonder how the annual changes in temperature, such as those depicted in the graphs of Fig. 2 , affect human health and the susceptibility to environmental toxicants. In a temperate climate, exposure to seasonal variations is assumed to have no remarkable effect on human health but this is not necessarily true. Seasonal effects on the susceptibility to disease should prompt toxicologists and pharmacologists to consider how the sensitivity to a drug or toxicant can be affected by seasonal change.

Generally, a V-, U-, or J-shaped relation is found between temperature and human mortality /88–90/. In the Netherlands, Kunst et al. /83/ performed a thorough analysis of mortality data comprising a database of 324 .7 deaths per day from 1979 to 1987 (Fig. 9). The authors showed a striking V-shaped relation between the air temperature and the mortality ratio. One cannot help but notice the remarkable similarity between the pattern shown in Fig. 9 and a typical thermoneutral profile of the

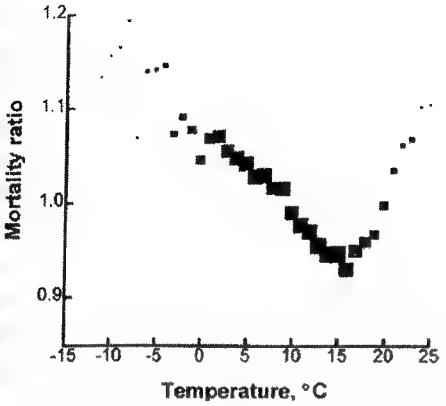

Fig. 9: Relation between average air temperature and daily mortality in humans. The data represent mortality rates (324.7 deaths per day) in the Netherlands from 1979 to 1987. The size of each block is proportional to the sample size. Data and graph modified from Kunst et al. /83/.

metabolic rate for a homeotherms—that is, as the ambient temperature increases or decreases from the thermoneutral zone, a zone associated with a minimal metabolic rate, bounded by the lower and upper critical temperatures associated with an elevation in metabolic rate. One would expect the seasonal effects on mortality to be dependent on latitude with respect to annual changes in temperature. A review of the mortality data in selected northern and southern cities in the eastern United States (U.S.) from 1973 to 1994 /88/ revealed a relation between temperature and mortality similar to that shown in Fig. 9. Cold-induced mortality had a greater impact on those living in warm, southern cities, whereas heat-induced deaths were more numerous in individuals living in northern cities. A comparison between Chicago and Miami illustrates how acclimatization to upper or lower latitudes can affect temperature-mortality relative risk functions. Chicago residents experienced respective mean summer and winter temperatures of 71.9° (21°C) and 25.6°F (3°C) compared with Miami temperatures of 82.3° (27.9°C) and 68.7°F (20.4°C). The estimated lower threshold temperature of the temperature-mortality function was 42°F (5.6°C) for Chicago and 71°F (21.6°C) for Miami. The upper threshold temperature was 75.5°F (24.2°C) for Chicago and 81.9°F (27.2°C) for Miami.

The significant relation b etween temperature and mortality is remarkable when one considers the uncontrolled biotic and abiotic variables in these studies. Most subjects would be expected to be sheltered much of the time from heat and cold stress, whereas such variables as humidity, level of physical activity (fitness), and diet would also affect mortality. A wide range in genotypes, socio-economic status, susceptibility to disease, and possible exposure to environmental contaminants could affect these functions.

Although soldiers and athletes represent young, healthy populations that do not have pre-existing physical ailments due to aging, significant hospitalization and deaths from exertion-related heat illness have been recorded for this group. Among such cases, most have occurred during the hottest summer months (typically July) and are likely a consequence of intense exercise associated with a lack of heat acclimatization. Obesity also appears to be a significant factor in heat mortality /91/. Given the increasing incidence of obesity in the U.S., this aspect may be a variable to consider in future toxicological studies.

From the above discussion, clearly, ambient temperature can have a profound influence on health and mortality, especially in the aging population. One can only wonder if exposure to environmental toxicants, as well as to certain drugs and other treatments, could factor into mortality curves like the one depicted in Fig. 9. That is, in view of the tremendous variability in biotic and abiotic factors that have a role in human health as described above, subtle effects of climate can exist that can be detected only with very sensitive analytical methods. For example, a Toronto study on the role of heat stress and pollution on human mortality detected a small but significant effect of air pollution on heat stress-related mortality /92/.

Frank and Tankersley /93/ recently developed a hypothesis to explain the link between the fluctuation in ambient PM (particulate matter)

pollution and human deaths, based on an age-related decline in thermoregulation and other homeostatic processes. Using telemetric monitoring of Tc and the electrocardiogram in the aging mouse, the authors suggested that sudden death from exposure to PM pollutants is related to an increase in the susceptibility of an individual as the ability to regulate Tc and heart rate declines with aging. Variations in ambient temperature and other climatic factors will also have a critical role in the ability to maintain a normal Tc and heart rate with aging. If mortality is a harbinger of other serious health effects of pollutants and high temperatures, then one can only assume that adding exercise and/or work to these conditions would increase the likely-hood of serious health effects that are linked to thermoregulation.

Geographic location can also have a significant impact on the interaction between toxicants and heat stress. The inhabitants of urban dwellings are exposed to a greater intensity and a longer duration of heat exposure because concrete structures do not effectively dissipate heat when nighttime temperatures decrease /94/. Furthermore, temperatures, whose magnitude is dependent on city size, are generally ~0.5°C to –1.0°C warmer in the city than those in rural areas, with 'urban heat islands' representing the warmest areas, typically in the center of the city. This information relates to recent findings from Stott et al. /95/ demonstrating that activities that increase the production of greenhouse gases will double the risk for extreme climate fluctuations. Thus, as man's activities increase in urban centers, the observation that more heat deaths are reported and expected in these areas is not surprising /96–97/.

Military Protective Clothing

In several occupations, wearing protective clothing can predispose individuals to the combined effects of toxicants and heat stress. Under normal clothing conditions, sufficient heat exchange between the skin surface and the environment can occur to regulate T_c within a narrow range, thus supporting thermal homeostasis. Protective clothing, which can consist of multiple layers and often encapsulates the head (a site of significant heat exchange /98/), forms an insulative layer of air between the skin and the environment, thus impeding heat exchange. The combination of an air layer that is estimated to be as large as 50L for industrial protective clothing /99/ and clothing constructed of multiple layers means that metabolically generated heat is required to pass through several microenvironments, each with their own insulative and vapor resistant properties, before exchange with the macroenvironment /99–100/.

Several studies examined the effect of nuclear, biological, or chemical protective clothing (NBC) on heat tolerance. The degree of heat stress experienced by the wearer of NBC clothing is dependent on environmental conditions. Wearing protective clothing, even at relatively low ambient temperatures of ~23°C, results in significant thermoregulatory and physiological stress, even at mild work intensities, thus reducing thermal tolerance /101–102/. Wearing NBC clothing during a treadmill exercise induced a ~15 percent increase in metabolic rate and T_c compared with subjects wearing normal clothing /103–105/. The absorption or trapping of sweat by the clothing effectively limits sweat evaporation at the skin surface, thus limiting heat dissipation. Although acclimation appears to reduce the core and skin temperature and the heart rate of normally clothed subjects, in subjects wearing protective clothing, the additional sweat secretion associated with acclimation is ineffective due to the inability of the sweat to penetrate the clothing. Thus, the acclimated subject experiences increased physical discomfort with no change in heat tolerance and a greater decrease in blood volume /105/.

Under conditions in which protective clothing leads to heat stroke, many physiological changes associated with this condition (hyperventilation, vasodilation, hypermetabolism) can exacerbate the effect of a toxicant. During deployment to arid

regions, military personnel can be exposed to thermal extremes whose effects are exacerbated under conditions requiring the wearing of NBC protective clothing. In addition to the potential effects of nerve agents, the antidotes for these substances often have adverse side effects of their own. In soldiers wearing chemical protective clothing, Kobrick and Johnson /106/ examined the side effects of a nerve agent antidote, atropine/2-PAM chloride (atropine/2-PAM chloride versus saline placebo) when combined with heat exposure at 95°F/60 percent relative humidity (RH) versus 70°F/30 percent RH. In soldiers wearing chemical protective clothing who were treated with the nerve antidote during exposure to 95°F (35°C), more adverse reactions were noted than in those wearing a battle dress uniform and receiving the placebo at 70°F (21°C). Whereas the adverse effects of protective clothing can predispose individuals to toxic insult, should it occur, one also must consider the potential for breakdown in the protective barrier of the clothing that might lead to toxicant/chemical exposure during the heat episode. In addition to enhancing a toxicant's effect, an increase in thermal stress while wearing protective clothing can impair cognition, resulting in an increased risk of accidents.

Acetylcholine (ACh) is a neurotransmitter in the central nervous system (CNS) in many organisms, including humans. Once released from the brain, a neurotransmitter binds to remote cell surface ACh receptors. After stimulating nerve fibers to contract, ACh is quickly removed by the enzyme acetylcholinesterase (AChE). Inhibition of enzyme activity results in an accumulation of excess ACh at the receptor site, causing excessive cholinergic stimulation. The consequent overstimulation of cholinergic pathways leads to a variety of sequelae that are characteristic of cholinergic poisoning, such as tremor and reduced motor activity, often leading to respiratory/cardiovascular failure. Body temperature can change occur as well.

Pyridostigmine is a reversible AChE inhibitor that is routinely used prophylactically in military personnel to protect against potential chemical warfare threats. Typical adverse effects associated with pyridostigmine are perturbations in peripheral nervous system functions because under normal conditions, the drug cannot cross the blood-brain barrier (BBB). Pyridostigmine, widely used in the Persian Gulf War, is suspected to have contributed to the Gulf War Syndrome, characterized by such symptoms as weakness, fatigue, headache, memory loss, and increased susceptibility to infections /107–109/. In Gulf War veterans, the ability of stress to increase BBB permeability has been suggested as a reason for the reported neurological and neuropsychological symptoms associated with pyridostigmine use. Under chemical warfare conditions, military personnel are also exposed to additional stress from the use of protective clothing.

Friedman et al. /107/ showed a central effect of pyridostigmine on AChe activity in mice exposed to mild stress. The stress-induced disruption of the BBB was shown by Evans Blue penetration into the brain parenchyma. Nevertheless, a controversy exists regarding the effects of stress on Evans Blue penetration as others have shown problems with using this technique in the rodent /110/. Furthermore, stressors used in such rodent studies are not necessarily representative of the types of stressors experienced by Gulf War veterans, making study interpretation to the human condition difficult. Heat stress represents a more realistic stressor to examine responses to pyridostigmine in the Gulf War Syndrome.

Any given neurotransmitter can bind to various subtypes of ACh receptors, each having distinct drug-binding and functional properties and distribution within the brain. The effects of ACh acting at different receptor subtypes are diverse. Henderson et al. /111/ exposed rats to subclinical levels of sarin in the presence or absence of heat stress and determined changes in cholinergic receptor subtypes. Sarin alone reduced cholinergic receptor subtypes in several areas of the brain, and under conditions of heat stress, the changes were extended to other areas of the brain.

TABLE 3

Incidence of clinical manifestations in 70 patients treated for acute exposure to organophosphate and carbamate insecticides (from Saadeh et al., 1996).

Clinical Manifestation	Number of Patients	Percentage
Muscarinic		
Miosis	60	89
Nausea	51	73
Salivation and bronchial constriction	51	73
Diarrhea/urinary incontinence	17	24
Nicotinic		
Muscular twitching	31	44
Tremor	9	13
Central nervous system		
Headache/dizziness	44	63
Coma	20	29
Cardiac		
Sinus tachycardia	25	36
Sinus bradycardia	20	28
Hypertension	15	22
Hypotension	12	17
Other		
Fever	34	49

The conclusion was that when sarin exposure occurs concomitantly with heat stress, the effects of levels that would not be clinically recognizable alone results in the delayed development of brain alterations in cholinergic receptor subtypes. Several of these effects under heat stress conditions extend to the hippocampus, an important area for memory function. The authors conclude that the combined effects of heat stress and sarin exposure on the CNS can explain the type of memory loss and cognitive dysfunction associated with the Gulf War Syndrome.

ANTI-CHOLINESTERASE FEVER: A COMMON THERMOREGULATORY RESPONSE IN RATS AND HUMANS

The incidence of clinical manifestations in 70 patients treated for acute exposure to OP and carbamate insecticides is presented in Table 3. The T_c of rodents as a benchmark of toxicity has been used frequently by toxicologists and pharmacologists since the 1950s. In a study of the anti-ChE agents in rodents, hypothermia, a decrease in T_c to

94°F (34.4°C) or lower, was considered the primary thermoregulatory response. Hypothermia is indeed the initial thermoregulatory response measured following relatively large doses of anti-ChEs because this parameter is easy to detect in rodents by conventional colonic probes. In addition, the T_c drop is marked, occurring over a relatively brief period (namely, < 6 hours). On the other hand, the most common thermoregulatory response of humans who are accidentally exposed to anti-ChE insecticides is fever (Table 3).

In one of the first reports of a fever-like response, Namba et al. /112/ found that humans exposed to the OP insecticide parathion could show sustained fever for at least 1 week after exposure. Similar findings of delayed fever reported in human cases of heat stroke indicate potentially overlapping mechanisms in the manifestation of this thermoregulatory response to toxicants and heat exposure. In humans, anecdotal evidence suggests that fever is a symptom of heat stroke, persisting for 7 to 14 days in some patients following clinical presentation /91, 113/. These observations suggest that fever, which is a tightly controlled physiological response to stress, is more directly related to the complications ensuing after heat and toxicant exposure than to the initial insult. Other investigators have shown that fever is the predominant response in humans, but occasionally a hypothermic response to toxicants is observed in the emergency room and typically treated with some type of heat source (for review, see /2/). The supposed opposite thermoregulatory responses between humans and rats to anti-ChEs would lead one to question the use of the rodent as a model for studying the health effects of such insecticides.

The development of radiotelemetry over the past 25 years has revolutionized the way in which physiological processes like T_c can be monitored in undisturbed laboratory rodents. Telemetry has shown that rats exposed to anti-ChE insecticides such as diisopropylfluorophosphate, chlorpyrifos, diazinon, and carbaryl undergo a delayed elevation in daytime T_c beginning 1 day after exposure and

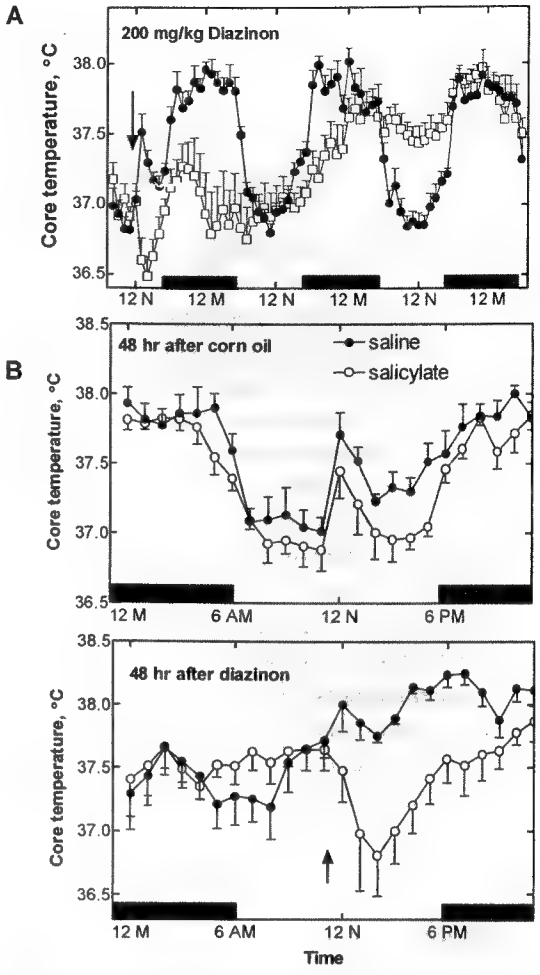

Fig. 10: **A.** Time course of core temperature in rats dosed with corn oil vehicle or the OP insecticide diazinon. **B.** Effect of sodium salicylate on T_c in rats dosed 48 hours before exposure to diazinon. (Data from Gordon & Mack /114/).

persisting in some cases for several days /2, 48/ (see Fig. 10A). Biomedical telemetry is a special area of biomedical instrumentation that permits the transmission of physiologic information from an often inaccessible location to a remote monitoring site. Although the telemetry of information can be done via telephone lines, most information is carried via radio link. Radiotelemetry allows one to detect subtle changes in a certain parameter that

develop over the course of several days or longer. Until the advent of telemetry, studying the time course of thermoregulatory changes in rodents was hampered by the effects of the stress of handling and of temperature measurements on the change in T_c in control and treated animals.

Such a rise in temperature is similar to that occurring during an infectious fever in many ways. The temperature elevation is seen primarily during the day when the T_c of control animals is normally reduced. During the fever, no compensatory tail vasodilation occurs to dissipate core heat, and rats that are allowed to thermoregulate behaviorally do not prefer cool temperatures A unique aspect of hyperthermia is that the response is blocked by the administration of antipyretics. The response of rats given sodium salicylate 48 hours after exposure to diazinon exemplifies the efficacy of an antipyretic given to rats dosed with an OP insecticide (Fig. 10C). Apparently, ra ts do not use autonomic or behavioral thermoeffectors to increase heat loss and to lower core temperature during the period of the OP-induced fever. On the other hand, the massive increase in circulating levels of interleukin-6, a white-blood-cell product characteristic of an infectious fever, has not yet been observed in rats subjected to a chlorpyrifos-induced fever (see /2/ for review). Fever represents the integrative responses of the immune and the thermoregulatory systems to mediate a regulated elevation in core temperature. The fever seen in humans and rats exposed to the anti-ChE insecticides provides toxicologists with a common benchmark that could be extrapolated from experimental animals to humans to study the mechanisms of this response.

CONCLUSIONS

Environmental conditions can have a profound impact on the physiological responses elicited in response to toxicant exposure. Unfortunately, toxicological studies are typically performed under standard laboratory conditions that are inappropriate for the maintenance of thermal homeostasis in small laboratory rodents, the most commonly used species for such studies. Housing at ambient temperatures below the thermoneutral zone imposes large metabolic demands on an organism for the maintenance of thermal homeostasis; such conditions are expected to have adverse consequences on the ability to respond to toxicant and/or heat exposure.

Hypothermia is the most common thermoregulatory response of mice and rats to toxicant and heat exposure, but the applicability of this response to the human condition is unknown. Hypothermia is not widely observed in human cases of environmental chemical and heat toxicity. Is this dissimilarity a consequence of differences in body mass between rodents and humans or does it speak to the inappropriateness of the laboratory conditions that have typically been used to examine the mechanisms of such responses in small rodents?

The thermoregulatory response to toxicant or heat exposure or both can have a profound impact on the ability of an organism to survive the insult. The protective effects of hypothermia and fever in survival from such environmental insults as glucose deprivation, hemorrhage, and hypoxia (oxygen deficiency) are well recognized, whereas the roles of these common thermoregulatory responses in protection against toxicant and heat exposure are not as well understood.

As reviewed in this paper, the biological efficacy of several toxicants is exacerbated under conditions of high ambient temperature. Yet, little is known regarding the mechanisms mediating the pathophysiological responses to the combination of these stressors. As exposure to environmental toxicants and drugs often occurs under stressful environmental conditions like high ambient temperature, a more thorough understanding of the mechanisms mediating the interaction between toxicants and heat exposure is required to develop effective therapeutic strategies for the mitigation of the harmful effects of these conditions.

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Approved for public release: distribution is unlimited. In conducting the research described in this report, the investigators adhered to the *Guide for Care and Use of Laboratory Animals*, as prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, U.S. National Research Council.

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